## CHIR0312.ST25 SEQUENCE LISTING

<110> Pizza, Mariagrazia Fontana, Maria Rita Giannelli, Valentina Rappuoli, Rina

<120> Immunogenic Detoxified Mutants Of Cholera Toxin

<130> CHIR0312

<150> 08/981,208

<151> 1997-12-22

<150> 9513371.6

<151> 1995-06-30

<160> 8

<170> PatentIn version 3.0

<210> 1

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<212> DNA

<213> Artificial Sequence

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<221> misc\_feature

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aattcatcag gcacaatcac a

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ggcagattct agacctcctg atgaaataaa

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tgaagtttgg cgaagcttct taatttgcca tactaattgc g

41

<210> 4

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<212> DNA

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<400> 4 aatgctccag gctcatcgat g

21

<210> 5

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<213> E. coli

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Asn Asp Phe Phe Arg Ala Asp Ser Arg Thr Pro Asp Glu Ile Arg Gln
1 5 10 15

Ala Gly Gly Leu Leu Pro Arg Gly Gln Gln Glu Ala Tyr Glu Arg Gly
20 25 30

Thr Pro Ile Asn Ile Asn Leu Tyr Glu His Ala Arg Gly Thr Val Thr 35 40 45

Gly Asn Thr Arg His Asn Asp Gly Tyr Val Ser Thr Thr Val Thr Leu 50 60

Arg Gln Ala His Leu Ile Gly Gln Asn Ile Leu Gly Ser His Asn Glu 65 70 75 80

Tyr Tyr Ile Tyr Val Val Ala Pro Ala Pro Asn Leu Phe Asp Val Asn 85 90 95

Gly Val Leu Gly Arg Tyr Ser Pro Tyr Pro Ser Glu Asn Glu Phe Ala  $100 \hspace{1.5cm} 105 \hspace{1.5cm} 110 \hspace{1.5cm}$ 

Ala Leu Gly Gly Ile Pro Leu Ser Gln Ile Ile Gly Trp Tyr Arg Val 115 120 125

Ser Phe Gly Ala Leu Glu Gly Gly Met Gln Arg Asn Arg Asp Tyr Arg 130 135 140

Gly Asp Leu Phe Ser Gly Leu Thr Val Ala Pro Asn Ala Asp Gly Tyr 145 150 155 160

Gln Leu Ala Gly Phe Pro Ser Asn Phe Pro Ala Trp Arg Glu Met Pro
165 170 175

Trp Ser Thr Phe Ala Pro Glu Gln Cys Val Pro Asn Asn Lys Glu Phe 180 185 190

Lys Ser Gly Val Cys Ile Ser Ala Thr Asn Val Leu Gly Lys Tyr Asp

Leu Met Asn Phe Lys Lys Leu Leu Lys Arg Arg Leu Ala Leu Thr Phe 210 215 220

Phe Met Ser Asp Asp Asp Phe Thr Gly Val His Gly Glu Lys Asp Glu 225 230 235 240

Leu

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<210> 6 <211> 236 <212> PRT <213> E. coli

<400> 6

Asn Asp Asp Lys Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile 1 5 10 15

Lys Gln Phe Arg Ser Leu Met Pro Arg Gly Ser Glu Tyr Phe Asp Arg 20 25 30

Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr Gln 35 40 45

Thr Gly Phe Val Arg His Asp Asp Gly Tyr Val Ser Thr Ser Ile Ser 50 55 60

Leu Arg Ser Ala His Leu Val Gly Gln Tyr Ile Leu Ser Gly His Ser 65 70 75 80

Leu Thr Ile Tyr Ile Val Ile Ala Asn Met Phe Asn Val Asn Asp Val 85 90 95

Ile Ser Ala Tyr Ser Pro His Pro Asp Glu Gln Glu Val Ser Ala Leu 100 105 110

Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr Arg Val His Phe 115 120 125

Gly Val Leu Asp Glu Gln Leu His Arg Asn Arg Gly Tyr Arg Asp Arg 130 135 140

Tyr Tyr Ser Asn Leu Asp Ile Ala Pro Ala Ala Asp Gly Tyr Gly Leu 145 150 155 160

Ala Gly Phe Pro Pro Glu His Arg Ala Trp Arg Glu Glu Pro Trp Ile 165 170 175

His His Ala Pro Pro Gly Cys Gly Asn Âla Pro Arg Ser Ser Met Ser 180 185 190

Asn Thr Cys Asp Glu Lys Thr Gln Ser Leu Gly Val Lys Phe Leu Asp

Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser Gly Tyr Gln Ser 210 215 220

Asp Ile Asp Thr His Asn Arg Ile Lys Asp Glu Leu 225 230 235

<210> 7

<211> 240

<212> PRT

<213> E. coli

<400> 7

Asn Gly Asp Arg Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile 1 5 10 15

Lys Arg Ser Gly Gly Leu Met Pro Arg.Gly His Asn Glu Tyr Phe Asp Page 3

 Arg
 Gly
 Thr 35
 Gln
 Met
 Asn
 Ile
 Asn Leu Tyr Asp His Ala Arg Arg Gly
 Thr Asp His Ala Arg Gly
 Thr Ser Leu Gly

 Gln
 Thr 50
 Gly
 Phe Val Arg Tyr Asp Asp Gly
 Tyr Val Ser Thr Ser Leu 60
 Ser Leu Arg Ser Ala His 70
 Leu Ala Gly Gln Ser 75
 Ile Leu Ser Gly His 80

 Ser Thr Tyr Tyr Ile 85
 Tyr Val Ile Ala Thr 90
 Ala Pro Asn Met 95
 Asn 95

 Val Asn Asp Val 100
 Gly Val Tyr Ser Pro His Pro Tyr Glu Gln Glu 110

Val Ser Ala Leu Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr 115 120 125

Arg Val Asn Phe Gly Val Ile Asp Glu Arg Leu His Arg Asn Arg Glu 130 135 140

Tyr Arg Asp Arg Tyr Tyr Arg Asn Leu Asn Ile Ala Pro Ala Glu Asp 145 150 155 160

Gly Tyr Arg Leu Ala Gly Phe Pro Pro Asp His Gln Ala Trp Arg Glu 165 170 175

Glu Pro Trp Ile His His Ala Pro Gln Gly Cys Gly Asp Ser Ser Arg 180 185 190

Thr Ile Thr Gly Asp Thr Cys Asn Glu Glu Thr Gln Asn Leu Ser Thr . 195 200 205

Ile Tyr Leu Arg Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser 210 215 220

Asp Tyr Gln Ser Glu Val Asp Ile Tyr Asn Arg Ile Arg Asp Glu Leu 225 230 235 240

<210> 8

<211> 240

<212> PRT

<213> Vibrio cholerae

<400> 8

Asn Asp Asp Lys Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  $1 \hspace{1.5cm} 5 \hspace{1.5cm} 10 \hspace{1.5cm} 15$ 

Lys Gln Ser Gly Gly Leu Met Pro Arg Gly Gln Ser Glu Tyr Phe Asp  $20 \hspace{1cm} 25 \hspace{1cm} 30$ 

Arg Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr 35 40 45

Gln Thr Gly Phe Val Arg His Asp Asp Gly Tyr Val Ser Thr Ser Ile 50 55 60

Ser Leu Arg Ser Ala His Leu Val Gly Gln Thr Ile Leu Ser Gly His 65 70 75 80

Ser Thr Tyr Tyr Ile Tyr Val Ile Ala Thr Ala Pro Asn Met Phe Asn  $85 \\ 90 \\ 95$ 

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Val	Asn	Asp	Val 100	Leu	Gly	Ala	Tyr	Ser 105	Pro	His	Pro	Asp	Glu 110	Gln	Glu
Val	Ser	Ala 115	Leu	Gly	Gly	Ile	Pro 120	Tyr	Ser	Gln	Ile	Tyr 125	Gly	Trp	Tyr
Arg	Val 130	His	Phe	Gly	Val	Leu 135		Glu	Gln	Leu	His 140	Arg	Asn	Arg	Gly
Tyr 145	Arg	Asp	Arg	Tyr	Tyr 150	Ser	Asn	Leu	Asp	Ile 155	Ala	Pro	Ala	Ala	Asp 160
Gly	Tyr	Gly	Leu	Ala 165	Gly	Phe	Pro	Pro	Glu 170	His	Arg	Ala	-	Arg 175	Glu
Glu	Pro	Trp	Ile 180	His	His	Ala	Pro	Pro 185	Gly	Cys	Gly	Asn	Ala 190	Pro	Arg
Ser	Ser	Met 195	Ser	Asn	Thr	Cys	Asp 200	Glu	Lys	Thr	Gln	Ser 205	Leu	Gly	Val
Lys	Phe 210	Leu	Asp	Glu	Tyr	Gln 215	Ser	Lys	Val	Lys	Arg 220	Gln	Ile	Phe	Ser
Gly 225	Tyr	Gln	Ser	Asp	Ile 230	Asp	Thr	His	Asn	Arg 235	Ile	Lys	Asp	Glu	Leu 240